Research main goal

In our research, we aim to investigate the potential of phytocannabinoids, specifically cannabidiol (CBD) and extracts from the non-psychoactive varieties of the medicinal hemp plant Cannabis sativa, in conjunction with modulation of the endocannabinoid system (ECS), to attenuate the process of NETosis within the tumor microenvironment.

Research description

Netosis is a defense mechanism employed by neutrophils, which are key components of the immune system. Neutrophils, serving as the body's first line of defense, migrate to sites of inflammation and utilize NETosis, among other processes, to eliminate microorganisms, necrotic cells, and cancer cells. NETosis involves the decondensation of chromatin, leading to the loosening of DNA structure and the release of extracellular DNA networks from the cell. This mechanism results in the lytic death of neutrophils, rendering them incapable of performing other critical functions such as phagocytosis or degranulation. The extracellular DNA networks released during NETosis are densely coated with various proteins and enzymes, which can degrade the extracellular matrix and potentially cause tissue damage. This process is particularly significant within the tumor microenvironment, characterized by chronic inflammation and extensive neutrophil recruitment. Cancer cells release chemotactic factors that enhance neutrophil migration and NETosis, resulting in the release of substantial quantities of DNA traps and proteolytic enzymes. These enzymes may facilitate the detachment of cancer cells from the tumor stroma, thereby promoting metastasis.

1. In the initial phase of our research, we aim to investigate whether cannabidiol (CBD), medicinal hemp extract, and modulation of the endocannabinoid system (via inhibition or activation of its receptors) can mitigate neutrophil NETosis induced by three distinct stimuli: phorbol 12-myristate 13-acetate (PMA), commonly utilized as an inducer of suicidal NETosis; conditioned media derived from cancer cell cultures; and a custom-prepared cocktail of factors secreted by cancer cells. Neutrophils will be isolated from the peripheral blood of healthy donors. We will employ Flow Cytometry and Western blot analysis to examine the prepared samples.

2. In the subsequent phase of the study, we intend to assess whether the attenuation of NETosis through the application of CBD or hemp extract and modulating the ECS system (via inhibition or activation of its receptors) impacts the pro-metastatic behavior of breast cancer cells. Specifically, we will co-incubate neutrophils with breast cancer cells in the presence of our selected NETosis-reducing compounds. Utilizing RT-qPCR, we will then evaluate whether the regulation of NETosis correlates with the expression profiles of genes we have identified as characteristic of cancer cells with high metastatic potential.

Rationale

Current research indicates that both the phytocannabinoid cannabidiol (CBD) and medicinal hemp extract, as well as modulation of the endocannabinoid system, can attenuate neutrophil NETosis in inflammatory contexts. However, these findings pertain to various disease models excluding cancer and its microenvironment. Furthermore, the precise mechanisms through which phytocannabinoids and the endocannabinoid system mitigate NETosis and excessive neutrophil activation remain inadequately elucidated. Our proposed research addresses an unexplored niche, focusing on a novel problem. Investigating this area could pave the way for developing effective therapies aimed at limiting neutrophil NETosis within the tumor microenvironment, thereby potentially reducting or even inhibiting cancer metastasis.

Anticipated Key Outcomes

We anticipate that the application of cannabidiol (CBD) or medicinal hemp extract, in conjunction with modulation of the ECS system, will attenuate the NETosis process primarily induced by conditioned media and factors secreted by breast cancer cells. Furthermore, we expect that this reduction in NETosis will correlate with a decrease in the expression levels of genes associated with metastasis. The outcomes of our research are expected to form a foundational basis for further investigation into this novel research problem, potentially leading to the development of therapeutic strategies aimed at mitigating neutrophil NETosis within the tumor microenvironment and thereby inhibiting cancer metastasis.